DETAILED ACTION

Status of the Claims

1. Claims 23-30 and 32 are pending.

Applicants' response and Declaration of Dr. Nigel J. Mouncey filed February 11, 2008 are acknowledged. Applicants' response and Declaration of Dr. Nigel J. Mouncey have been fully considered. Therefore, claims 23-30 and 32 are examined.

Withdrawn Claim Rejections - 35 USC § 112

2. The previous rejection of claims 25 and 26 under 35 U.S.C. 112, first paragraph, scope enablement and written description, is withdrawn in view of applicant's response at pages 8-15 of the remark and Declaration of Dr. Nigel J. Mouncey filed February 11, 2008.

3. The previous rejection of claims 23 and 32 under 35 U.S.C. 112, second paragraph, is withdrawn in view of applicant's response at pages 5-8 of the remark filed February 11, 2008.

While the specification indicates the term "mutation" refers to alteration in the genomic sequence of the microorganism, for clarity of the claim, it is suggest that claim 23, step (b) can be amended to recite "introducing a mutation causing a biotin auxotrophy into the genomic sequence of microorganism……target fermentation product".

New Claim Objections

4. Claim 24 is objected to because of the use of the term "A process according to claim 23". Since claim 24 is dependent from claim 23, it is suggested to use "The process according to claim 23". The same type of objection is also applied to claims 25-29.

Maintained Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 23-24 and 32 are rejected under 35 U.S.C. 112, first paragraph because the specification, while being enabling for a process for decoupling production of a specific target fermentation product (i.e., riboflavin) from biomass production in a fermentation medium, the method comprising: (a) providing a recombinantly produced microorganism of bacillus that contains a polynucleotide sequence which encodes biosynthetic enzymes for the target fermentation product (i.e., riboflavin), (b) introducing a mutation causing a biotin auxotrophy into a specific gene of the microorganism such as bioFDB gene cassette (e.g., SEQ ID NO:1) to control biomass production, and (c) supplying the medium with unlimited amount of substrates for producing the riboflavin and with a limited amount of biotin complementing the auxotrophy; and a microorganism made by the process, does not reasonably provide enablement for a process for decoupling production of a target fermentation product (i.e., riboflavin) from biomass production in a fermentation medium, the method comprising: (a) providing a recombinantly produced microorganism of bacillus that contains a polynucleotide sequence which encodes biosynthetic enzymes for the target fermentation product (i.e., riboflavin), (b) introducing a mutation causing biotin auxotrophy into the microorganism to control biomass production, and (c) supplying the medium with unlimited amount of substrates for producing the riboflavin and with a limited amount of biotin complementing the auxotrophy; and a microorganism made by the process, where the mutated gene causing biotin auxotrophy is not identified. The specification does not enable a person skilled in the art to which it pertains, or with which it is

most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Claims 23-24 and 32 are directed to a process for decoupling production of a target fermentation product from biomass production in a fermentation medium using a recombinantly produced microorganism of bacillus comprising a polynucleotide sequence which encodes biosynthetic enzymes for the target fermentation product, and introducing a mutation causing a biotin auxotrophy into the microorganism; and a microorganism made by the process, wherein the target fermentation product is riboflavin. The specification, however, only discloses cursory conclusions without data supporting the findings (pages 5-6), which state that the present invention provides a process for decoupling production of a target fermentation product from biomass production in a fermentation medium. This process includes providing a recombinantly produced microorganism that has been engineered to contain a polynucleotide sequence which encodes the biosynthetic enzymes for a target fermentation product, where the maximal production of the target fermentation product is dependent on an unlimited supply of a target fermentation product substrate for the microorganism; and then an auxotrophy is introduced into the microorganism to control biomass production by limiting the concentration of a substrate complementing the auxotrophy in the fermentation medium; and a fermentation production microorganism made by the process. There are no indicia that the present application enables the full scope of the claims in view of the claimed method as discussed in the stated rejection. The present application does not provide sufficient teaching/guidance to enable the full scope of the claims. The factors considered in determining whether undue experimentation is required, are summarized in In re Wands (858 F2d at 731,737, 8 USPQ2d at 1400,1404 (Fed. Cir.1988)). The

factors most relevant to this rejection are the breadth of the claims, the presence or absence of working examples, the state of the prior art and relative skill of those in the art, the predictability or unpredictability of the art, the nature of the art, the amount of direction or guidance presented, and the amount of experimentation necessary.

(1). The breadth of the claims:

The breadth of the claims is broad and encompasses unspecified variants regarding the mutated genes in the microorganism that cause biotin auxotrophy, which are not adequately described or demonstrated in the specification.

(2). The absence or presence of working examples:

While the specification describes introducing a specific biotin auxotroph mutant construct comprising SEQ ID NO:1 (a polynucleotide comprising deletion-insertion in *bioFDB* gene cassette) into a riboflavin production microorganism RB50 containing multiple copies of pRF69, culturing fermentations, and measuring biomass and riboflavin production at different biotin concentrations (see Examples 1-3), the specification has not identified various mutated genes in the microorganism that cause biotin auxotrophy as encompassed by the claims, and there is no structure/activity correlation for various mutated genes.

(3). The state of the prior art and relative skill of those in the art:

The related art (references on pages 1-4 of the specification) teach recombinant production of riboflavin and genes involved in the riboflavin biosynthetic pathways; and the art (e.g., Bower et al. U.S. Patent 6,303,377; Bower et al. J. Bacteriology 178, 4122-4130 (1996)) shows the genes of the biotin biosynthetic operon of *Bacillus subtitis*; and insertion and deletion in the specific genes of bio operon that cause biotin auxotrophy. However, the art does not

disclose various mutated genes that cause biotin auxotrophy other than specific mutated genes in the bio operon. Since the general knowledge and level of the skill in the art do not supplement the omitted description (i.e., various mutated genes that cause biotin auxotrophy as encompassed by the claims) the specification needs to provide additional teachings on identification of various mutated genes that cause biotin auxotrophy other than specific mutated genes in the bio operon.

(4). Predictability or unpredictability of the art:

The claims encompass a process for decoupling production of a target fermentation product (i.e., riboflavin) from biomass production in a fermentation medium using a recombinantly produced microorganism of bacillus comprising a polynucleotide sequence which encodes biosynthetic enzymes for the target fermentation product, and introducing a mutation causing a biotin auxotrophy into the microorganism; and a microorganism made by the process. Since the specification merely discloses introducing a specific biotin auxotroph mutant construct comprising SEQ ID NO:1 (a polynucleotide comprising deletion-insertion in *bioFDB* gene cassette) into a riboflavin production microorganism RB50 that causes biotin auxotrophy, and there is no information on other various mutated genes that cause biotin auxotrophy, thus the identities of various mutated genes that cause biotin auxotrophy other than those in bio operon are unpredictable.

(5). The amount of direction or guidance presented and the quantity of experimentation necessary:

The claims are directed to a process for decoupling production of a target fermentation product (i.e., riboflavin) from biomass production in a fermentation medium using a recombinantly produced microorganism of bacillus comprising a polynucleotide sequence which

encodes biosynthetic enzymes for the target fermentation product, and introducing a mutation causing a biotin auxotrophy into the microorganism; and a microorganism made by the process. The specification describes introducing deletion-insertion mutations within a bioFDB gene cassette of Bacillus subtilis such as a specific biotin auxotroph mutant construct comprising SEQ ID NO:1 into a riboflavin production microorganism RB50 containing multiple copies of pRF69, culturing fermentations, and measuring biomass and riboflavin production at different biotin concentrations (see Examples 1-3). However, the specification has not identified any other mutated genes that cause biotin auxotrophy as encompassed in the claims. Moreover, there are no working examples demonstrating the use of various recombinantly produced microorganisms transformed with various mutated genes other than those in bio operon that cause biotin auxotrophy. Since the specification does not provide sufficient teachings on various mutated genes that cause biotin auxotrophy in the recombinantly produced microorganisms of bacillus, it is necessary to carry out undue experimentation to identify the mutated genes that cause biotin auxotrophy from numerous mutated genes.

(6). Nature of the Invention

The scope of the claim encompasses a process for decoupling production of a target fermentation product from biomass production in a fermentation medium using a recombinantly produced microorganism of a bacillus and introducing a mutation causing biotin auxotophy into the microorganism, but the specification does not provide sufficient teachings on the identities of various mutated genes that cause biotin auxotrophy. Thus, the disclosure is not enabling for the reasons discussed above.

In summary, the scope of the claim is broad, the working example does not demonstrate the claimed method associated with variants, the teachings in the specification are limited, and the identities of biotin auxotrophy-causing genes are unpredictable, and therefore, it is necessary to carry out undue experimentation to identify the mutated genes causing biotin auxotrophy.

Response to Arguments

In the Declaration of Dr. Nigel J. Mouncey, paragraph 6 summarizes the claimed invention; paragraph 7 states that claims 23-26 and 32 have been rejected for lack of enablement and lack of written description under 35 U.S.C. § 112, first paragraph; paragraphs 8-10 state the Examiner's reasoning for enablement and written description rejections; paragraphs 11-13 state Bower *et al.* (J. Bacteriology 178, 4122-4130 (1996); Exhibit 1) disclose that *bio* genes of *B subtilis* are located in a single operon and that genes with similarity to *bioW, bioA, bioF, bioD,* and *bioB* are found in this operon (page 4122), that the construction of various *Bacillus subtilis* mutants which require biotin for their normal growth (pages 4127-28), that insertion and deletion mutations within the *Bacillus subtilis* bio operon and flanking DNA (page 4127 and Fig. 4), and that the location of these mutations (pages 4127-28 and Table 4); paragraph 14 states Sasaki *et al.* (Biosci. Biotechnol. Biochem. Vol. 68, No. 3, pp. 739-742 (2004); Exhibit 2) disclose the introduction of mutants into the biotin operon leading to an auxotrophy (pages 740-41), for example, a BioW gene mutation, which results in an opal stop codon, and a deletion mutation in the BioF gene, and the location of these mutations (Fig. 1); paragraph 15 states these documents confirm that generating biotin auxotrophic mutants as described and claimed in the instant application was within the skill in the art, and the level of knowledge and skill in this art is high; and paragraphs 16-21 state that the genes involved in biotin biosynthesis are well known to those

skilled in the art, and the specification discloses that the mutation causing auxotrophic growth may be introduced using any convenient means, such as by chemical and UV mutagenesis followed by screening or selection for a desired phenotype (p. 8, lines 16-19), where simple screens for confirming an auxotrophy are disclosed (p. 12, lines 18-20), and a specific exemplification of a process for decoupling production of riboflavin from biomass production with biotin auxotrophy, including a description of how to make a specific biotin auxotroph (p.15-18; Examples 1-3; and Figs. 1-4). Screening large numbers of mutants is simply a matter of routine lab work that does not require inordinate skill. Moreover, in this art there is no need to identify structure/activity correlations because the bio operon was well known and characterized, manipulating different genes within the operon to suit one's purpose was well within the skill of the art when the present application was filed. Thus, based on the information disclosed in the specification, the knowledge of the structure of the biotin operon and the genes contained therein, a person skilled in the art at the time the present invention was made and who was familiar with the knowledge in the art, would be able to make and use the claimed invention.

Applicant indicates claim 23 currently recite (1) that the recombinantly produced microorganism is a Bacillus, (2) that biotin is the specific auxotrophy, and (3) that riboflavin is the specific target fermentation product; and a Declaration of Dr. Nigel J. Mouncey has been submitted to demonstrate the claimed process is fully enabled that a person skilled in the art would readily be able to make and use the claimed invention. Applicant further asserts that in the Declaration, Dr. Mouncey discusses two articles (Bower et al. (1996) and Sasaki et al. (2004)) that disclose the introduction of mutations into the biotin operon, which lead to, *e.g.,* biotin auxotrophs (Declaration, ¶¶ 11-15). Dr. Mouncey further states that the genes involved in

biotin biosynthesis are well known to those skilled in the art, and the specification discloses that the mutation causing auxotrophic growth may be introduced using any convenient means, such as by chemical and UV mutagenesis followed by screening or selection for a desired phenotype (p. 8, lines 16-19), where simple screens for confirming an auxotrophy are disclosed (p. 12, lines 18-20), and a specific exemplification of a process for decoupling production of riboflavin from biomass production with biotin auxotrophy, including a description of how to make a specific biotin auxotroph (p.15-18; Examples 1-3; and Figs. 1-4). As Dr. Mouncey's declaration clearly conveys, one skilled in the art, with the present application in hand would only be required to carry out routine experiments to make and identify other biotin auxotrophs. Thus, in view of the clear disclosure in the specification of how to make biotin auxotrophs that produce riboflavin, and the acknowledged high degree of skill and knowledge in the art, the claims are sufficiently enabled. Therefore, the rejection should be withdrawn (pages 8-12 of the response).

Applicant's response and Declaration of Dr. Nigel J. Mouncey have been fully considered. Regarding claims 25-26, directed to the step of introducing a polynucleotide comprising deletion-insertion mutations within a bioFDB cassette into the microorganism, the arguments are persuasive, and the rejection is withdrawn. However, regarding claims 23-24 and 32, the arguments are not persuasive because of the following reasons. While the genes involved in biotin biosynthesis are known in the art, a convenient means may be used to introduce a mutation in the genes involved in biotin biosynthesis, and a screening method may be used to confirm a biotin auxotrophy, the claimed method recites the step (b) of introducing a mutation causing a biotin autotrophy into the microorganism to control biomass, in which the gene to be mutated is not identified, and the number of possible mutated genes to be tested is virtually

endless. Besides the specific mutated genes in the bio operon show biotin auxotrophy as indicated in Bower *et al.* (J. Bacteriology 178, 4122-4130 (1996)), there are no other mutated genes causing biotin auxotrophy described at the time of filing of the instant application. Furthermore, the claimed method does not recite the steps of introducing a mutation causing auxotrophic growth using any convenient means, such as by chemical and UV mutagenesis followed by screening or selection for a desired phenotype, as argued by the applicants, but instead, the claimed method recites the step of introducing a mutation causing biotin auxotrophy into the microorganism without identifying the gene to be mutated that causing a biotin auxotrophy. Thus, in reading the claims, a skilled person would not know which gene would be chosen for mutation to result in biotin auxotrophy in addition to the specific mutated genes in the bio operon. Therefore, it is necessary to have additional guidance regarding the identity of the mutated genes and to carry out undue experimentation to identify the mutated genes that cause biotin auxotrophy. Thus, the full scope of the claims are not enabled.

6. Claims 23-24 and 32 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 23-24 and 32 are directed to a process for decoupling production of a target fermentation product from biomass production in a fermentation medium using a recombinantly produced microorganism of bacillus comprising a polynucleotide sequence which encodes biosynthetic enzymes for the target fermentation product, and introducing a mutation causing a

biotin auxotrophy into the microorganism, wherein the target fermentation product is riboflavin; and a microorganism made by the process.

In *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1938, the Court of Appeals for the Federal Circuit has held that "A written description of an invention involving a chemical genus, like a description of a chemical species, requires a precise definition, such as by structure, formula, [or] chemical name, of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP § 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

While the specification indicates that the invention provides a process for decoupling production of a target fermentation product from biomass production in a fermentation medium by introducing a specific biotin auxotroph mutant construct comprising SEQ ID NO:1 into *bacillus subtilis* RB50 containing multiple copies of the engineered *rib* operon pRF69, culturing fermentations, and measuring biomass and riboflavin production at different biotin concentrations, which shows the product yield (i.e., the amount of riboflavin produced on the

consumed glucose) is 33% higher in the decoupled process to the coupled process (see Examples 1-3), the specification does not disclose a genus of variants for mutated genes that cause biotin auxotrophy in a transformed microorganism as encompassed by the claims. Introducing a single species of a mutated gene (i.e., SEQ ID NO:1) into the microorganism (RB50::[pRF69]Bio$^-$ transformed with SEQ ID NO:1 at different biotin concentration to produce riboflavin; Example 3) does not provide written description for the genus of variants of mutated genes that cause biotin auxotrophy in the claimed method, which would encompass identifying mutated genes causing biotin auxotrophy from numerous mutated genes. There is no way to predict the identity of the mutated gene that would cause biotin auxotrophy other than specific mutated genes in bio operon (Bower et al. 1996) due to lack of information in the various genes causing biotin auxotrophy. Without guidance on the structures of various mutated genes that cause biotin auxotrophy, one skilled in the art would not know the identities of the mutated genes that cause biotin auxotrophy. The lack of description on the structures of the mutated genes that cause biotin auxotrophy, and the lack of representative species as encompassed by the claims, applicants have failed to sufficiently describe the claimed invention, in such full, clear, concise terms that a skilled artisan would not recognize applicants were in possession of the claimed invention.

Response to Arguments

Applicant indicates claim 23 currently recite (1) that the recombinantly produced microorganism is a Bacillus, (2) that biotin is the specific auxotrophy, and (3) that riboflavin is the specific target fermentation product; and a Declaration of Dr. Nigel J. Mouncey has been submitted to demonstrate that the claimed process is described in the specification in

such a way to reasonable convey to one skilled in the art that Applicants were in possession of the claimed invention as of the filing date. Applicant further asserts that in the Declaration, Dr. Mouncey discusses two articles (Bower et al. (1996) and Sasaki et al. (2004)) that disclose the introduction of mutations into the biotin operon, which lead to, *e.g.,* biotin auxotrophs (Declaration, ¶¶ 11-15). Dr. Mouncey further states that the specification discloses that the mutation causing auxotrophic growth may be introduced using any convenient means, such as by chemical and UV mutagenesis followed by screening or selection for a desired phenotype (p. 8, lines 16-19), where simple screens for confirming an auxotrophy are disclosed (p. 12, lines 18-20), and a specific exemplification of a process for decoupling production of riboflavin from biomass production with biotin auxotrophy, including a description of how to make a specific biotin auxotroph (p.15-18; Examples 1-3; and Figs. 1-4). As Dr. Mouncey's declaration indicated that the level of skill in the art, the well known structure and organization of the biotin operon, including how to make and identify such auxotrophs, and the extensive disclosure in the specification that there was not need to identify structure/function correlation in order to demonstrate possession of the claimed invention. Thus, in view of Dr. Mouncey's opinion, the extensive disclosure in the specification, and the acknowledged high degree of skill and knowledge in the art, the applicants were in possession of the full scope of the claimed invention (pages 12-14 of the response).

Applicant's response and Declaration of Dr. Nigel J. Mouncey have been fully considered. Regarding claims 25-26, directed to the step of introducing a polynucleotide comprising deletion-insertion mutations within a bioFDB cassette into the microorganism, the arguments are persuasive, and the rejection is withdrawn. However, regarding claims 23-24 and

32, the arguments are not persuasive because of the following reasons. While the genes involved in biotin biosynthesis are known in the art, a convenient means may be used to introduce a mutation in the genes involved in biotin biosynthesis, and a screening method may be used to confirm a biotin auxotrophy, the claimed method recites the step (b) of introducing a mutation causing a biotin autotrophy into the microorganism to control biomass, in which the gene to be mutated is not identified, thus the number of possible mutated genes to be tested is virtually endless. Furthermore, the claimed method does not recite the steps of introducing the mutation causing auxotrophic growth using any convenient means, such as by chemical and UV mutagenesis followed by screening or selection for a desired phenotype as argued by applicant, but instead, the claimed method recites step of (b) without identifying the gene to be mutated that causing a biotin auxotrophy. Since numerous mutated genes can be introduced into microorganism, and there is no structure to function/activity correlation established for the mutated genes, a skilled person would not know how to choose a proper mutated genes that cause biotin auxotrophy other than the specific mutated genes in bio operon as indicated in Bower (1996). Without description on the identification of various mutated genes that cause biotin auxotrophy other than the specific mutated genes in bio operon, and the lack of representative species as encompassed by the claims, a skilled artisan would not recognize applicants were in possession of the claimed invention. Therefore, the rejection is maintained.

Conclusion

7. Claims 23-24 and 32 are rejected; and claims 25-30 are objected to.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Chih-Min Kam whose telephone number is (571) 272-0948. The examiner can normally be reached on 8.00-4:30, Mon-Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Bragdon can be reached at 571-272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Chih-Min Kam/
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CMK
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